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(54) CORPS LIPIDIQUES SERVANT D'EXCIPIENT TOPIQUE POUR DES AGENTS ACTIFS
(54) OIL BODIES AS TOPICAL DELIVERY VEHICLES FOR ACTIVE AGENTS

(57)

[222]The present invention relates to the topical delivery of active agents, for [2]example cosmetic compounds, to living organisms. In particular the present [2]invention relates to the administration of therapeutically active agents in [2]the presence of compositions obtainable from living cells known as oil bodies. [2]The oil bodies of the present invention are especially suitable in [2]applications where it is desired that the active agent is topically delivered. [2]The oil bodies of the present invention are preferably obtained from oil seed [2]plants.[2]

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(57) Abrégé/Abstract:
The present invention relates to the topical delivery of active agents, for example cosmetic compounds, to living organisms. In particular the present invention relates to the administration of therapeutically active agents in the presence of compositions obtainable from living cells known as oil bodies. The oil bodies of the present invention are especially suitable in applications where it is desired that the active agent is topically delivered. The oil bodies of the present invention are preferably obtained from oil seed plants.

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(54) Title: OIL BODIES AS TOPICAL DELIVERY VEHICLES FOR ACTIVE AGENTS

(57) Abstract

The present invention relates to the topical delivery of active agents, for example cosmetic compounds, to living organisms. In particular the present invention relates to the administration of therapeutically active agents in the presence of compositions obtainable from living cells known as oil bodies. The oil bodies of the present invention are especially suitable in applications where it is desired that the active agent is topically delivered. The oil bodies of the present invention are preferably obtained from oil seed plants.

Title: Oil Bodies as Topical Delivery Vehicles for Active Agents

FIELD OF THE INVENTION

The present invention relates to novel compositions and methods for the delivery of active agent to animals including humans. The compositions comprise oil bodies and an active agent. The compositions are particularly useful for the topical delivery of the active agents.

BACKGROUND OF THE INVENTION

Active ingredients such as pharmaceutical actives can be administered to the body via a number of routes including ingestion, injection, and topical application. Regardless of the route of delivery, the active ingredient must overcome certain barriers such as biological membranes, for example the stratum corneum in the skin, before the agent can exert the desired biological effect at its target site in the body. Various techniques have been investigated for the potential to enhance transport and delivery of active agents to their target site and numerous systems that facilitate the delivery of active agents are known to the prior art.

Among the most extensively evaluated delivery systems for actives are those systems permitting delivery of active agents into the skin. Active ingredients exerting a biological effect on the skin include actives capable of mitigating wrinkles, reducing hyperpigmentation, treating UV damaged skin cells and the like. Approaches to facilitate topical delivery of these active agents include the use of physical forces such as ultrasound and electricity as well as the use of chemical delivery systems to enhance penetration of the active ingredient into the skin (see: Chen, L.-H. and Chien, Y.W. Enhancement of skin penetration. In: Novel Cosmetic Delivery Systems. Marcel Dekker, Inc., New York, pp 51-69.).

Chemical delivery vehicles can be divided into three broad types of delivery vehicles: vesicular, porous polymeric and particulate delivery vehicles. Vesicular delivery vehicles include, liposomes, niosomes, and transfersomes. Liposomes are lipid vesicles typically from about 30 to 100 nm in diameter, composed of one or more lipid bilayers surrounding an aqueous interior. The traditional liposome is constructed using phopholipids like phosphatidyl choline and have more recently been constructed using single-chain amphiphiles or nonionic surfactants (niosomes). In general, liposomes are manufactured in a four-step process. The first step involves the mixing of amphiphilic molecules in organic solvents, the stirring of the mixture, the separation of the solvent by the addition of water to cause the detachment of phospholipid bilayers and the homogenization to mechanically form particles of a specified size and homogeneity. Transfersomes are special lipid aggregates formed by the mixture of amphiphilic substances and active ingredients and subjecting them to filtration, ultrasonication, stirring, agitating or any other mechanical fragmentation (EP 0 475 160 B1).

Porous particulate delivery vehicles include web-like systems and porous-sphere systems. In the web-like system, an "empty" polymer can have an active ingredient loaded onto it or a nanoparticle can be preloaded with an active ingredient. In the porous-sphere system a porous membrane surrounds a solid nanoparticle. These systems are formed using cross-linked polymers, for example substituted acrylates (Nacht S. 1995. Cosmetics & Toiletries 110: 25-30).

The final type of delivery vehicle is the particulate delivery system. Examples of particulate delivery vehicles include microcapsules, beads and microspheres. Microcapsules are analogous to the shell of an egg. They have multilayer construction with multiple cores containing the active. The classic microcapsule is constructed using gelatin, cellulose-type polymers or synthetic polymers. Beads and microspheres are small solid partices (for example nylon). The active ingredient is adsorbed onto the particle for later delivery (Romanowski P and Schueller R. 1999. Stability Testing of Cosmetic Products. In: Novel Cosmetic Delivery Systems. Marcel Dekker, Inc., New York, pp 115-130).

However despite considerable efforts to develop delivery systems for active ingredients, the available delivery vehicles are frequently inefficient. There are two important reasons for the limited effectiveness of the currently available delivery technology. First, the delivery systems known to the prior art display a lack of permeability through the biological barriers. Second, the applied active ingredient even if it is capable of permeating the biological barrier, does not migrate to the active site. Thus the effectiveness of delivery is compromised due to distribution of the active ingredient to non-target tissues or cells. Additionally this may result in undesirable side-effects, for example cosmetic actives may cause irritating reactions. Furthermore, the active ingredient may be metabolized prior to reaching the target. In order to compensate for the dilution of the active agent, higher doses than otherwise are desirable must be administered.

In the seeds of oilseed plants, which include economically important crops such as rapeseed and sunflower, the oil fraction is stored in discrete subcellular structures variously known to the art as oil bodies, oleosomes, lipid bodies or spherosomes (Huang, 1992, Ann. Rev. Plant Mol. Biol. 43: 177-200). Besides a mixture of oils, also referred to in the chemical art as triacylglycerides, oil bodies comprise phospholipids and a number of associated proteins collectively termed oil body proteins. From a structural point of view, oil bodies are substantially spherical structures which encompass a matrix comprising a mixture of lipids encapsulated by a phospholipid monolayer and oil body proteins. The predominant protein present in the oil body is known as oleosin (Huang, 1992, Ann. Rev. Plant Mol. Biol. 43: 177-200). Intact oil bodies have been isolated previously from a variety of oil seed crops, see for example: Jacks et al., JAOCS, 67: 353-361 and Huang, 1992, Ann. Rev. Plant Mol. Biol. 43: 177-200. In general, the objective of this experimental work

has been to elucidate the *in vivo* structure and/or function of oil bodies in the seed. In order to obtain plant oils for industrially relevant applications, for example for use in cosmetics or detergents, seeds are generally crushed, pressed and subsequently refined. Since the objective of these processes is to obtain pure plant oil, the oil bodies in the course of the production process lose their structural integrity.

United States patents 5,683,740 and 5,613,583 disclose emulsions comprising lipid vesicles that have been prepared from crushed oleagenous plant seeds. In the course of the crushing process, oil bodies substantially lose their structural integrity. Accordingly, these patents disclose that in the crushing process, 70% to 90% of the seed oil is released in the form of free oil. Thus the emulsions which are the subject matter of these patents are prepared from crushed seeds from which a substantial amount of free oil has been released while the structural integrity of the oil bodies is substantially lost. In addition, the emulsions disclosed in both of these patents are prepared from relatively crude seed extracts and comprise numerous endogenous seed components including glycosylated and non-glycosylated non-oil body seed proteins. It is a disadvantage of the emulsions to which these patents relate that they comprise contaminating seed components imparting a variety of undesirable properties, which may include allergenicity and undesirable odor, flavor, color and organoleptic characteristics, to the emulsions. Due to the presence of seed contaminants, the emulsions disclosed in these patents have limited applications.

There is need in the art to provide improved delivery vehicles and methods by which active agents can be effectively topically delivered to living organisms.

SUMMARY OF THE INVENTION

The present invention relates to the use of oil bodies as improved topical delivery vehicles for active agents. The present inventors have shown that by delivering an active agent in an oil body formulation the absorption or penetration of the active agent is enhanced. Further, certain active agents that are generally irritable to the skin have reduced irritability when delivered in an oil body formulation as compared to other formulations such as safflower oil or liposomes or when administered alone.

The present invention provides a composition for the improved delivery to the skin of an active agent to a living organism wherein the composition comprises:

(1) an active agent; and

(2) oil bodies.

The present invention further provides a use of a composition comprising an active agent and oil bodies to deliver an active agent to a living organism. The present invention also includes a use of oil bodies to prepare a medicament or composition to deliver an active agent to a living organism.

The present invention further provides a method of preparing a composition for the delivery of an active agent which comprises formulating active agent in the presence of oil bodies to prepare a composition.

The present invention provides a method for the delivery of an active agent to a living organism comprising administering an effective amount of a composition comprising the active agent and oil bodies to the skin of an organism in need thereof.

The active agents that may be used in accordance with the present invention including without limitation cosmeceuticals, cosmetically active agents and dermatological pharmaceuticals. The active agent is topically administered to the living organism.

In a preferred embodiment, the active agent is a cosmetically active agent such as hydroquinone, salicylic acid, and tretinoin, which are formulated in the presence of oil bodies, applied topically and delivered percutaneously to the skin. Preferably, the absorption or penetration of the cosmetically active agent in the oil body formulation is enhanced and the irritability is reduced as compared to the penetration or absorption and irritability of the active agent in the absence of the oil bodies. Accordingly, the compositions of the invention may be used in improved topical formulations to beautify the skin or to treat skin conditions.

In another embodiment of the invention, the active agent is a protein which is produced recombinantly on the surface of the oil body. The oil bodies are then formulated and administered to the living organism.

Additional advantages and features of the present invention will become apparent after consideration of the accompanying drawings and the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a Coomassie blue stained gel of washed oil body preparations obtained from white mustard, oilseed rape (*Brassica napus*), soybean, peanut, squash, flax, sunflower, safflower and maize.

Figure 2 is a Western Blot analysis of human skin fractions wherein the stratum corneum was removed prior to the application of oil bodies in a Franz assay. Western analysis of epidermal fractions (50 µg /lane) from 3 individuals (lanes 3 through 5) and dermal fractions (45 µg /lane) from the same 3 individuals (lanes 6 through 8) are compared to controls (50 µg epidermal extract spiked with 0.2 µg of canola oil body protein (lane 1) and 50 µg control intract skin (lane 2)). The anti-oleosin antibody used in this experiment was a polyclonally-derived antibody to an canola oil body.

Figure 3 is a Western Blot analysis of human skin fractions wherein the stratum corneum was removed after the application of oil bodies and the completion of the Franz assay. Western analysis of epidermal fractions (50 µg /lane) from 3 individuals (lanes 2

through 4) and dermal fractions (50 µg /lane, 45 µg /lane, 40 µg /lane respectively) from the same 3 individuals (lanes 5 through 7) are compared to controls (50 µg epidermal extract spiked with 0.2 µg of canola oil body protein (lane 1) and 50 µg /lane control intract skin (lane 2)). The anti-oleosin antibody used in this experiment was a polyclonally-derived antibody to an canola oil body.

Figure 4 is a histogram representing the cumulative results of 7 strips in a skin-stripping assay for formulations containing salicyclic acid, hydroquinone and tretinoin. Compared are the oil body formulations containing the active ingredient or a simple emulsion placebo containing the active ingredient. The cumulative percentage recovery of active ingredient obtained from 7 strips is indicated.

DETAILED DESCRIPTION OF THE INVENTION

A. Compositions

As hereinbefore mentioned, the present invention provides improved compositions for the enhanced delivery of active agents to living organisms. The compositions comprise an active agent in an oil body formulation.

The present inventors have unexpectedly discovered that oil bodies have many features that render them especially suitable as versatile delivery vehicles of active compounds. Importantly, the inventors have shown that by delivering an active agent in an oil body formulation the absorption or penetration of the active agent to the skin is enhanced. Further, certain active agents that are generally irritable to the skin have shown to have reduced irritability when delivered in an oil body formulation as compared to other formulations such as safflower oil or liposomes. Therefore, the oil bodies are extremely mild when administered to the skin and beneficial in that they mask the irritating effect of known cosmetic active ingredients, such as salicylic acid and hydroquinone. In addition, the creamy texture of the oil body compositions renders these compositions especially suitable for the preparation of delivery vehicles that are dermatologically acceptable. Furthermore, a wide variety of active ingredients can easily be formulated using oil bodies and oil bodies can be prepared on a large scale using known seed milling equipment combined with a simple aqueous extraction process. Oil bodies are preferably obtained from plant sources which have GRAS (Generally Regarded As Safe) status. The oil body based formulations therefore can be safely used to topically deliver active agents.

Accordingly, in one aspect, the present invention provides a composition for the topical delivery of an active agent which comprises:

(1) an active agent; and

(2) oil bodies.

The term "active agent" or "agent" as used herein means any agent that one would like to administer to a living organism. The term includes without limitation any

agents capable of mediating an improvement or benefit to the physical appearance, health, fitness or performance of the surface area of the body. In preferred embodiments of the invention the active agent is a cosmetically active agent, a cosmeceutical, or a dermatologically active pharmaceutical active agent. The active agent may be obtained from any suitable source or synthesized chemically.

The terms "cosmetically active agent" or "cosmeceutical" are used interchangeably throughout this application and denote ingredients that have a desirable biological effect on external or superficial surfaces of a living organism, preferably a human, more preferably the skin, hair, teeth and nails of the human. The cosmeceutical may be used to beautify the skin or to treat skin conditions, diseases or abnormalities. For example, the cosmetically active agent may be vitamin A, C or E, alpha hydroxy acids, such as citric, glycolic, lactic and tartaric acid, exfoliating agents such as salicylic acid, bleaching agents such as hydroquinone, anti-aging compounds and sunscreens such as octyl methoxycinnamate (Parsol MCX), 3-benzophenone (Uvinul M40) and butylmethoxydibenzoylmethane (Parsol 1789), or a compound that is capable of exerting an immunostimulatory effect such as a β-glucan. Cosmetically active agents also include enzymes for example papain, bromelin, ficin, lipases and proteases all of which have been used in the preparation of cosmeceuticals.

The terms "dermatologically active agent" or "dermatological agent" are used interchangeably throughout this application and refer to all compounds regulated as drugs, for example antibiotics, fungicides, antiviral agents, anti-inflammatory agents used to treat skin conditions or diseases.

Further examples of active agents that may be employed in accordance with the present invention, include but are not limited to amino acids, anticancer agents (carboplatin), antimetabolite methotrexate (MTX), azidothymidine, ceramide, corticosteroids (for example halicinonide, tiramcinolone acetonide, betamethasone valerate, etc.), cyclosporin, dextran, disinfectants, dithranol, econazole, estradiol, fibronectin, glial-derived neurotrophic factor (GDNF), glucocorticoids, hair growth stimulants, herbal drugs, lectins, local anesthetics, methotrexate, minioxidil, moisturizing agents, placenta hydrolysate, phospholipase C, pregnenolone, progesterone, prostaglandin, retinoids (vitamin A acid, isotretinoin), rubefacients, skin protecting agents (gelating hydrolysate, embryonic extract, aloe extract, vanillin), steroid hormones, terconazole, testosterone, tetracaine, thymus extract, tretinoin, triamcinolone acetonide (TRMA), tyrosine and its derivatives, trimcinolone acetonide, and urea.

The active agent may be linked to the oil bodies, either covalently or non-covalently. In embodiments of the instant invention where the active agent is a protein or peptide, one particularly advantageous way in which the biologically active ingredient may be included in the oil body preparation is through construction of oleosin gene fusions as

detailed in WO 96/21029 which is incorporated herein by reference. Accordingly, the present invention provides a composition for the delivery of a polypeptide of interest wherein the polypeptide is linked to a sufficient portion of an oleosin to provide targeting of the polypeptide to an oil body. Isolation of the oil body fraction results in recovery of the active agent attached to the oil bodies. In principle any desired protein or peptide may be produced using this technology.

Examples of therapeutic proteins may be used in accordance with the present invention include, but are not limited to enzymes such as papain, bromelin, ficin, lipases, collagenase, elastase and proteases, and proteins such as thioredoxin, collagen, elastin, or active fragments or derivatives of any of these proteins.

It is to be clearly understood that the particular active agent is not of critical importance and may be as desired. Accordingly it is to be clearly understood that in accordance with the present invention in principle the oil body preparation may be applied as a topical delivery vehicle for any active agent.

The term "living organism" refers to any living organism which includes all members of animal kingdom. In preferred embodiments, the living organism is a vertebrate, more preferably, a human.

The term "oil bodies" as used herein means a substantially intact discrete subcellular oil or wax storage organelle. The oil bodies may be obtained from any cell containing oil bodies or oil body-like organelles. This includes animal cells, plant cells, fungal cells, yeast cells (Leber, R. et al., 1994, Yeast 10: 1421-1428), bacterial cells (Pieper-Fürst et al., 1994, J. Bacteriol. 176: 4328 - 4337) and algae cells (Roessler, P.G., 1988, J. Phycol. (London) 24: 394-400). In preferred embodiments of the invention the oil bodies are obtained from a plant cell which includes cells from pollens, spores, seed and vegetative plant organs in which oil bodies or oil body-like organelles are present (Huang, 1992, Ann. Rev. Plant Physiol. 43: 177-200). More preferably, the oil body preparation of the subject invention is obtained from a plant seed and most preferably from the group of plant species comprising: rapeseed (*Brassica* spp.), soybean (*Glycine max*), sunflower (*Helianthus annuus*), oil palm (*Elaeis guineeis*), cottonseed (*Gossypium* spp.), groundnut (*Arachis hypogaea*), coconut (*Cocus nucifera*), castor (*Ricinus communis*), safflower (*Carthamus tinctorius*), mustard (*Brassica* spp. and *Sinapis alba*), coriander (*Coriandrum sativum*), squash (*Cucurbita maxima*), linseed/flax (*Linum usitatissimum*), Brazil nut (*Bertholletia excelsa*), jojoba (*Simmondsia chinensis*), maize (*Zea mays*), crambe (*Crambe abyssinica*) and eruca (*Eruca sativa*). In order to obtain oil bodies, plants are grown and allowed to set seed using agricultural cultivation practises well known to a person skilled in the art. After harvesting the seed and if desired removal of material such as stones or seed hulls (dehulling), by for example sieving or rinsing, and optionally drying of the seed, the seeds are subsequently processed by mechanical pressing, grinding or crushing. A liquid phase

may be added prior to or while grinding the seeds. This is known as wet milling. Preferably the liquid is water although organic solvents such as ethanol may also be used. Wet milling in oil extraction processes has been reported for seeds from a variety of plant species including: mustard (Aguilar et al 1991, Journal of Texture studies 22:59-84), soybean (US Patent 3,971,856; Cater et al., 1974, J. Am. Oil Chem. Soc. 51:137-141), peanut (US Patent 4,025,658; US Patent 4,362,759), cottonseed (Lawhon et al., 1977, J. Am. Oil, Chem. Soc. 54:75-80) and coconut (Kumar et al., 1995, INFORM 6 (11):1217-1240). It may also be advantageous to imbibe the seeds for a time period from about fifteen minutes to about two days in a liquid phase prior grinding. Imbibing may soften the cell walls and facilitate the grinding process. Imbibition for longer time periods may mimic the germination process and result in certain advantageous alterations in the composition of the seed constituents. In another embodiment, the liquid phase is added after the seeds are ground. This is known as dry milling. Preferably the added liquid phase is water.

The seeds are preferably ground using a colloid mill, such as the MZ130 (Fryma Inc.). Besides colloid mills, other milling and grinding equipment capable of processing industrial scale quantities of seed may also be employed in the here described invention including: flaking rolls, disk mills, colloid mills, pin mills, orbital mills, IKA mills and industrial scale homogenizers. The selection of the mill may depend on the seed throughput requirements as well as on the source of the seed which is employed. It is of importance that seed oil bodies remain substantially intact during the grinding process. The term "substantially intact" as used herein means that the oil bodies have not released greater than 50% (v/v) of their total seed content in the form of free oil. Preferably, grinding of the seed therefore in the release of less than about 50% (v/v) of the total seed oil content, more preferably less than about 20% (v/v) and most preferably less than about 10% (v/v). Accordingly, in a preferred embodiment the present invention provides a composition for the improved delivery of an active agent comprising (a) an active agent and (b) substantially intact oil bodies.

Therefore, any operating conditions commonly employed in oil seed processing, which tend to disrupt oil bodies are unsuitable for use in the process of the subject invention. Milling temperatures are preferably between 10°C and 90°C and more preferably between 26°C and 30°C, while the pH is preferably maintained between 2.0 and 10.

Solid contaminants, such as seed hulls, fibrous material, undissolved carbohydrates and proteins and other insoluble contaminants, are removed from the crushed seed fraction. Separation of solid contaminants, may be accomplished using a decantation centrifuge, such as a HASCO 200 2-phase decantation centrifuge or a NX310B (Alpha Laval). Depending on the seed throughput requirements, the capacity of the decantation centrifuge may be varied by using other models of decantation centrifuges, such as 3-phase decanters. Operating conditions vary depending on the particular centrifuge which is

employed and must be adjusted so that insoluble contaminating materials sediment and remain sedimented upon decantation. A partial separation of the oil body phase and liquid phase may be observed under these conditions.

Following the removal of insoluble contaminants, the oil body phase is separated from the aqueous phase. In a preferred embodiment of the invention a tubular bowl centrifuge is employed. In other embodiments, hydrocyclones, disc stack centrifuges, or settling of phases under natural gravitation or any other gravity based separation method may be employed. It is also possible to separate the oil body fraction from the aqueous phase employing size exclusion methods, such as membrane ultrafiltration and crossflow microfiltration. In preferred embodiments the tubular bowl centrifuge is a Sharples model AS-16 (Alpha Laval) or a AS-46 Sharples (Alpha Laval). A critical parameter is the size of the ring dam used to operate the centrifuge. Ring dams are removable rings with a central circular opening varying, in the case of the AS-16, from 28 to 36 mm and regulate the separation of the aqueous phase from the oil body phase thus governing the purity of the oil body fraction which is obtained. In preferred embodiments, a ring dam size of 29 or 30 mm is employed when using the AS-16. The exact ring dam size employed depends on the type of oil seed which is used as well as on the desired final consistency of the oil body preparation. The efficiency of separation is further affected by the flow rate. Where the AS-16 is used flow rates are typically between 750-1000 ml/min (ring dam size 29) or between 400-600 ml/min (ring dam size 30) and temperatures are preferably maintained between 26°C and 30°C. Depending on the model centrifuge used, flow rates and ring dam sizes must be adjusted so that an optimal separation of the oil body fraction from the aqueous phase is achieved. These adjustments will be readily apparent to a skilled artisan.

Separation of solids and separation of the aqueous phase from the oil body fraction may also be carried out concomitantly using a gravity based separation method such as 3-phase tubular bowl centrifuge or a decanter or a hydrocyclone or a size exclusion based separation method.

The compositions obtained at this stage in the process, generally are relatively crude and comprise numerous seed proteins, which includes glycosylated and non-glycosylated proteins and other contaminants such as glucosinilates or breakdown products thereof.

In preferred embodiments of the present invention, significant amount of seed contaminants are removed. To accomplish removal of contaminating seed material, the oil body preparation obtained upon separation from the aqueous phase is washed at least once by resuspending the oil body fraction and centrifuging the resuspended fraction. This process yields what, for the purpose of this application, is referred to as a washed oil body preparation. The number of washes will generally depend on the desired purity of the oil

body fraction. Preferably the washed oil bodies contain less than about 75% (w/v) of all endogenously present non-oil body seed proteins, more preferably less than about 50% (w/v) of non-oil body seed proteins and most preferably less than about 10% (w/v) of all endogenously present non-oil body seed proteins. Accordingly, in another embodiment, the present invention provides a composition for the improved delivery of an active agent comprising (a) an active agent; and (b) washed oil bodies, more preferably, substantially intact washed oil bodies.

Depending on the washing conditions which are employed, an essentially pure oil body preparation may be obtained. In such a preparation the only proteins present would be oil body proteins. In order to wash the oil body fraction, tubular bowl centrifuges or other centrifuges such hydrocyclones or disc stack centrifuges may be used. Washing of oil bodies may be performed using water, buffer systems, for example, sodium chloride in concentrations between 0.01 M and at least 2 M, 0.1 M sodium carbonate at high pH (11-12), low salt buffer, such as 50 mM Tris-HCl pH 7.5, organic solvents, detergents or any other liquid phase. In preferred embodiments the washes are performed at high pH (11-12). The liquid phase used for washing as well as the washing conditions, such as the pH and temperature, may be varied depending on the type of seed which is used. Washing at a number of different pH's between pH 2 and pH 11-12 may be beneficial as this will allow the step-wise removal of contaminants, in particular proteins. Washing conditions are selected such that the washing step results in the removal of a significant amount of contaminants without compromising the structural integrity of the oil bodies. In embodiments where more than one washing step is carried out, washing conditions may vary for different washing steps. SDS gel electrophoresis or other analytical techniques may conveniently be used to monitor the removal of seed proteins and other contaminants upon washing of the oil bodies. It is not necessary to remove all of the aqueous phase between washing steps and the final washed oil body preparation may be suspended in water, a buffer system, for example, 50 mM Tris-HCl pH 7.5, or any other liquid phase and if so desired the pH may be adjusted to any pH between pH 2 and pH 10. The oil bodies may be preserved by heat treatment for example by pasteurization in a constant temperature water bath at approximately 65°C for 20 minutes. The pasteurization temperature preferably ranges between 50°C and 90°C and the time for pasteurization preferably ranges between 15 seconds to 35 minutes.

The process to manufacture the oil body preparation may be performed in batch operations or in a continuous flow process. Particularly when tubular bowl centrifuges are used, a system of pumps generating a continuous flow. The pumps may be for example a 1 inch M2 Wilden air operated double diaphragm pumps or hydraulic or peristaltic pumps may be employed. In order to maintain a supply of homogenous consistency to the decantation centrifuge and to the tubular bowl centrifuge, homogenizers, such as an IKA

homogenizer may be added between the separation steps. In-line homogenizers may also be added in between various centrifuges or size exclusion based separation equipment employed to wash the oil body preparations. Ring dam sizes, buffer compositions, temperature and pH may differ in each washing step from the ring dam size employed in the first separation step.

B. Properties of Oil bodies

When viewed under the electron microscope, the oil bodies that are obtained are found to be more or less spherically shaped structures (see: Example Murphy, D. J. and Cummins I., 1989, Phytochemistry, 28: 2063-2069; Jacks, T. J. et al., 1990, JAOCS, 67: 353-361). Typical sizes of oil bodies vary between 0.4 µm for and 1.5 µm (Murphy, D. J. and Cummins I., 1989, Phytochemistry, 28: 2063-2069). When analyzed using a Malvern Size Analyzer, it was found that oil bodies in a washed oil body preparation isolated from rapeseed were symmetrically and unimodally distributed around 1 µm. Using a Malvern Size Analyzer a washed oil body preparation could be clearly distinguished from commercially obtainable oil-in-water emulsions including soymilk, mayonnaise (Kraft Real Mayonnaise) and two coconut milk preparations (Tosca, Aroy-D). The exact size and density of the oil bodies depends at least in part on the precise protein/phospholipid/triacylglyceride composition which is present.

The oil bodies present in the washed oil body preparations of the present invention are resistant to exposure to strong acids and bases, including prolonged exposure to acidic conditions at least as low as pH 2 and alkaline conditions at least as high as pH 10. When exposed to pH 12, a slight loss of oil was observed, indicating a loss of integrity of the oil body structure. In addition, extraction with various organic solutions, including methanol, ethanol, hexane, isopropyl alcohol and ethyl acetate, does not or only slightly compromise the integrity of the oil bodies present in the washed oil body preparation. The oil bodies present in the washed oil body preparation were also found to withstand mixing with the anionic detergent, sodium dodecyl sulfate (SDS), the cationic, detergent hexadecyl trimethyl bromide and Tween-80, a non-ionic detergent. Boiling of the washed oil body preparation in the presence of SDS was found to result at least partly in distntegration of the oil body structure. The oil bodies present in the washed oil body preparation are stable when maintained for 2 hrs up to at least 100°C. A slow freeze and thaw of washed oil body preparations resulted in a change in their physical appearance characterized by the formation of clumps as opposed to a homogeneous emulsion. Oil body clumping following a freeze-thaw could also be prevented to a large degree by either a) flash freezing in liquid nitrogen instead of slow freezing at -20°C or b) adding glycerol in excess of 5% (v/v) to the oil body preparation prior to freezing. The resistance to relatively harsh chemical and physical conditions, is a unique characteristic of the oil bodies present

in the washed oil body preparation of the subject invention and makes oil bodies uniquely suited as delivery vehicles.

For many applications, it is also considered desirable that a purer, better defined oil body preparation is obtained, as this allows more control over the formulation process of the final emulsion. In order for the washed oil body preparation to be included in a diverse set of final preparations it is desirable that volatiles are kept to a minimum and the colour is preferably light or white. Washing of the oil body preparation results in a lighter coloured preparation. In addition, a substantial amount of volatiles is removed. Also removed by washing are compounds which promote the growth of microorganisms as it was observed that a washed oil body preparation had a longer shelf life than an unwashed preparation. Other compounds which are removed by washing include anti-nutritional glucosinilates and/or breakdown products thereof and fibrous material. When heat treated to 60°C or 80°C, it was observed that larger quantities of water remained absorbed by the washed oil body preparation when compared with an unwashed preparation. Upon cooling down to room temperature and centrifugation, it was observed that the washed oil body preparation remained stable, while phase separation occurred in the unwashed preparation. Given the enhanced stability of washed oil bodies, they are preferred where the formulation process involves the application of heat. When heated to 40°C, the washed oil body preparation was able to absorb a larger quantity of exogenously added water without resulting in phase separation. Thus in the formulation of aqueous emulsions, washed oil bodies may be preferred. The capacity to absorb exogenously added oils was also compared between a preparation of washed oil bodies and an unwashed preparation. Larger amounts of exogenous oil could be added to the washed oil body preparation before an unstable emulsion was formed. This is advantageous in applications where exogenous oils or waxes are added in the formulation process such as in topical applications. When viscosity was compared between a washed oil body preparation and an unwashed preparation it was found that the washed preparation was more viscous. A more viscous preparation of oil bodies is desirable as this eliminates the need for the addition of thickening agents in the formulation process.

The above observations were made using oil body preparations obtained from rapeseed and prepared as detailed in Example 2 of the present application. It is believed that resistance to relatively harsh chemical and physical conditions will be a characteristic of the oil bodies present in the washed oil preparation of the subject invention regardless of the source of the oil bodies. However one or more of the hereinbefore documented properties for rapeseed oil bodies may vary depending on the living cell, plant species or the genetic line from which the washed oil bodies preparation is obtained. Nevertheless it is to be clearly understood that the subject invention is drawn

to an oil body preparation which may be obtained from any living cell comprising oil bodies.

In embodiments of the present invention where the oil bodies are obtained from non-seed cells, the oil body preparation is isolated following similar procedures as outlined above. In embodiments of the invention where the oil bodies are isolated from softer tissues, for example the mesocarp tissue of olives, the techniques applied to break open the cell may vary somewhat from those used to break harder seeds. For example, pressure-based techniques may be preferred over crushing techniques. The methodology to isolate oil bodies on a small scale has been reported for isolation of oil bodies from mesocarp tissues in olive (*Olea europaea*) and avocado (*Persea americana*) (Ross et al., Plant Science, 1993, 93: 203-210) and from microspore-derived embryos of rapeseed (*Brassica napus*) (Holbrook et al., Plant Physiol., 1991, 97: 1051-1058).

In embodiments of the invention where oil bodies are obtained from non-plant cells, the oil body preparation is isolated following similar procedures as outlined above. The methodology to isolate oil bodies from yeast has been documented (Ting et al., 1997, Journal of Biol. Chem. 272: 3699-3706).

The chemical and physical properties of the oil fraction may be varied in at least two ways. Firstly, different plant species contain oil bodies with different oil compositions. For example, coconut is rich in lauric oils (C12), while erucic acid oils (C22) are abundantly present in some Brassica spp. Secondly, the relative amounts of oils may be modified within a particular plant species by applying breeding and genetic engineering techniques known to the skilled artisan. Both of these techniques aim at altering the relative activities of enzymes controlling the metabolic pathways involved in oil synthesis. Through the application of these techniques, seeds with a sophisticated set of different oils are obtainable. For example, breeding efforts have resulted in the development of a rapeseed with a low erucic acid content (Canola) (Bestor, T. H., 1994, Dev. Genet. 15: 458) and plant lines with oils with alterations in the position and number of double bonds, variation in fatty acid chain length and the introduction of desirable functional groups have been generated through genetic engineering (Töpfer et al., 1995, Science, 268: 681-685). Using similar approaches a person skilled in the art will be able to further expand on the presently available sources of oil bodies. Variant oil compositions will result in variant physical and chemical properties of the oil bodies. Thus by selecting oilseeds or mixtures thereof from different species or plant lines as a source for oil bodies, a broad repertoire of oil body preparations with different textures and viscosities may be acquired.

In one embodiment of the present invention, the oil bodies are obtained from oil seeds. The presence of intact oil bodies in the emulsion and the described characteristics of

these oil bodies clearly distinguish the subject emulsion formulation from other materials which may be prepared from plant seeds.

C. Method of Preparing Composition

In another aspect, the present invention provides a method of preparing a composition for the delivery of an active agent which comprises formulating the active agent in the presence of oil bodies to prepare a composition.

The term "formulating" as herein used means any process that results in contacting oil bodies with the active agent.

The active agent may be incorporated into the oil body preparation in any desired manner. The active agent may be added as a solution, suspension, a gel or solid. The active agent may upon formulation become associated with the oil bodies, remain suspended in solution, or form a suspension in which the oil bodies are dispersed. The active agent may also penetrate the phospholipid monolayer surrounding the oil body or the triacylglyceride matrix. The active agent may be linked to the oil bodies in a non-covalent manner, in which case the active agent may be a protein as well as any other molecule. Methodologies for non-covalently linking active molecules to oil bodies are further detailed in US Patent 5,856,452 and WO 98/27115 both of which are incorporated by reference herein.

When the active agent is a protein, it may be prepared as a recombinant fusion protein with an oil body protein or oleosin as described in WO 96/21029 which is incorporated herein by reference. Accordingly, the present invention provides a method of preparing a composition for the delivery of an active agent comprising:

(a) introducing into a host cell a chimeric DNA sequence comprising:

(1) a first DNA sequence capable of regulating the transcription in said host cell of

(2) a second DNA sequence, wherein said second sequence encodes a recombinant fusion polypeptide and comprises a DNA sequence encoding a sufficient portion of an oleosin protein to provide targeting of the recombinant fusion polypeptide to an oil body phase, linked in frame to (ii) a DNA sequence encoding the active agent; and

(3) a third DNA sequence encoding a termination region functional in said host cell;

(b) growing said host cell to produce said recombinant fusion polypeptide

(c) separating the oil body fraction of said host cell from the cellular components to prepare oil bodies and the active agent.

The oil bodies containing the active agent may be formulated to prepare a composition.

The amount of active agent that will be employed will be that amount that will be necessary to deliver a desirable biological effect at the site of delivery. In particular, an effective amount depends, inter alia upon the particular active agent, the route of administration, and in the case of pharmaceutically active agents the severity of the condition or disorder under treatment and other factors. In general, the concentration of the active agent in the delivery system can vary from as little as 0.001% up to 50% by weight of the composition. More typically the active concentration is between about 0.01% and 10% by weight of the composition. Cell culture assays and animal model may be used to assist in determining doses appropriate for human if appropriate. Skilled artisans will be able to adjust the quantity of active in the composition.

A variety of additional ingredients may be included in the final composition that is administered to the living organism. In preferred embodiments these ingredients are added to formulate a dermatologically acceptable formulation for topical skin application. For example, water may be added either directly or through moisture associated with the therapeutically active agent. The final amount of water is not critical. Generally, the final delivered formulations will contain at least 1% of water and up to 99% water by weight. Usually mixing will be required to provide an adequate suspension and it may be necessary to apply heat or pressure or to change the pH.

The amount of the oil bodies and active agent in the final administered composition may vary considerably and can vary from as little as 0.1% to 99.9%. More typically however the oil bodies and active agent will comprise between 5% and 95% of the final administered composition.

In other embodiments an oil or a wax will be an additional ingredient. Oils or waxes may partition to the triacyl glyceride matrix of the oil bodies. Where oils or waxes comprise the added ingredient, the oil bodies may remain suspended in the lipophilic phase or double emulsions may be formed.

Generally, the compositions will be treated such that contamination by bacteria, fungi, mycoplasmas, viruses and the like or undesired chemical reactions, such as oxidative reactions are prevented thus allowing the preparation of a stable final product with a shelf-life accepetable for the final composition. In preferred embodiments this is accomplished by the addition of preservatives, for example sodium metabisulfite, Glydant Plus, Phenonip, methylparaben propylparaben, Germall 115, Germaben II, phytic acid, or other chemical additives, by irradiation, for example by ionizing radiation such as cobalt-60 or cesium-137 irradiation or by ultraviolet irradiation or by heat treatment for example by pasteurization in a constant temperature water bath at approximately 65°C for 20 minutes. The pasteurization temperature preferably ranges between 50 and 90°C and the

time for pasteurization preferably ranges between 15 seconds to 35 minutes. Oxidative reactions may be prevented by the addition of anti-oxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisol (BHA) or other anti-oxidants.

The physical stability of the compositions may be further enhanced if desired by the addition of an emulsifier such as for example Arlacel. Typically, emulsion stabilizers are added in small amounts (less than 2% by weight).

The final compositions may be in solid or in liquid form or of any other desired viscosity. The viscosity of the emulsion may be modified using a viscosity modifier such as cetyl alcohol. The emulsion may be thickened using gelling agents such as cellulose and derivatives, Carbopol and derivatives, carob, carregeenans and derivatives, xanthane gum, sclerane gum, long chain alkanolamides, bentone and derivatives, Kaolin USP, Veegum Ultra, Green Clay, Bentonite NFBC, typically present in concentrations less than 2% by weight. The composition may also form a coating or film.

The compositions may further comprise surfactants to wet, foam, penetrate, emulsify, solubilize and or disperse the cosmetically active agent. For example anionic surfactants such as sodium coconut monoglyceride sulphonate, cationic surfactants, such as lauryl trimethyl ammonium chloride, cetyl pyridinium chloride and trimethylammonium bromide, nonionic surfactants including pluronics, and polyethylene oxide condensates of alkyl phenols, and zwitterionic surfactants such as derivatives of aliphatic quaternary ammonium, phosmomium and sulphonium compounds may all be added as required. Chelating agents, capable of binding metal ions, such as tartaric acid, EDTA, citric acid, alkali metal citrates, pyrophosphate salts or anionic polymeric polycarboxylates may be also included in the final formulation as desired.

The compositions of the present invention may further comprise additional hydrocarbon compounds such as plant, animal, mineral or synthetic oils or waxes or mixes thereof. They comprise paraffin, petrolatum, perhydrosqualene, arara oil, almond oil, calphyllum oil, avocado oil, sesame oil, castor oil, jojoba oil, olive oil, or cereal germ oil. Esters may be included such as esters of lanolic acid, oleic acid, lauric acid, stearic acid, myristic acid. It is also possible to include alcohols for example, oleoyl alcohol, linoleyl alcohol or linolenyl alcohol, isostearyl alcohol or octyl dodecanol, alcohol or polyalcohol. Further hydrocarbons which may be included are octanoates, decanoates, ricinoleates, caprylic/capric triglycerides or C10 to C22 fatty acid triglycerides. The addition of these agents may result in the formation of double emulsions. Hydrogenated oils, which are solid at 25°C, such as hydrogenated castor oil, palm oil or coconut oil, or hydrogenated tallow; mono- di- tri- or sucroglycerides; lanolins; and fatty acids which are solid at 25°C may also be included in the topically applied formulations of the present invention. Among the waxes which may be included are animal waxes such as beeswax; plant waxes such as carnauba wax, candelilla wax, ouricurry wax, Japan wax or waxes from cork fibres or sugar

cane; mineral waxes, for example paraffin wax, lignite wax, microcrystalline waxes or ozokerites and synthetic waxes. Pigments may be included and may be white or coloured, inorganic or organic and/or paerlescent. These pigments comprise titanium dioxide, zinc oxide, ziriconium dioxide, black, yellow, red and brown iron oxides, cerium dioxide, chromium oxide, ferric blue, carbon black, barium, strontium, calcium and aluminum lakes and mica coated with titanium oxide or with bismuth oxide.

Moisturizing agents which may be included in topically applied compositions are for example mineral oil and urea. Antioxidants such as the naturally occurring tocopherols and polyphenols, or butylated hydroxytoluene and hydroxyanisole may also be also added.

While the final formulations or compositions may vary considerably in composition and contain numerous additional ingredients, in general the final compositions for topical applications may be formulated in accordance with methods used and known by those skilled in the art of formulating cosmetic and dermatological formulations.

D. Uses

The present invention includes all uses of the compositions of the invention to deliver active agents to the skin of an organism in need thereof. Accordingly, the present invention provides a use of a composition comprising an active agent and oil bodies to deliver an active agent to a living organism. The present invention also includes a use of a composition comprising oil bodies and an active agent to prepare a medicament to deliver an active agent to a living organism.

The present invention further provides a method for the delivery of an active agent to a living organism comprising administering an effective amount of a composition comprising the active agent and oil bodies to the skin of an organism in need thereof.

The term "living organism" as used herein includes all members of the animal kingdom. Preferably the living organism is a vertebrate, more preferably a mammal, most preferably a human.

The term "effective amount" as used herein means an amount effective, at dosages and for periods of time necessary to achieve the desired results.

The composition is preferably administered topically which includes, without limitation, administration to the skin, hair, teeth and nails.

Topical administration includes administration to the skin, including human or animal skin. The compositions of the present invention may be used to beautify the skin and to treat the skin for example hyperpigmented or hypopigmented skin, age spots and other skin changes associated with aging such as wrinkles, blotches and atrophy or elastotic skin changes characterized by leathery changes associated with intrinsic aging or skin damage caused by extrinsic factors such as sunlight radiation, X-ray radiation, air pollution, wind, cold, dampness, heat, smoke and cigarette smoking. Additional skin conditions which may

be treated with the delivery vehicles of the present invention include but are not limited to acne keratoses, palmar or plantar hyperkeratosis, psoriasis, eczema, seborrheic eczema, pruritus, ichthyosis, Darier's disease, lichen simplex chronicus, inflammatory dermatoses, basal cell carcinoma, squamous cell carcinoma, malignant cell carcinoma, and AIDS related Kaposi sarcoma.

When applied topically the active ingredient is delivered percutaneously. The term "percutaneous" as used herein refers to the delivery to the skin without referring to their eventual fate. Accordingly, the active ingredient may be delivered to various layers of the skin including the stratum corneum, the epidermis and the dermis.

When it is desirable to deliver the therapeutically active ingredient to the epidermis or to the dermis, the active may conveniently be linked to an oil body protein, for example an oleosin, through covalent or non-covalent bonds and topically applied. Based on the observation that oil body associated proteins penetrate into the epidermis and dermis (examples 4 and 5), the active ingredient linked to the oil body should be delivered in the epidermal and dermal layers of the skin.

Upon administration of the formulation to the living organism, the active ingredient may remain associated with the oil body and exert its biological effect, for example when a formulation is used which has been prepared by covalently or non-covalently linking the active ingredient to the oil body. It is also possible that the active ingredient separates from the oil body prior to exerting its biological effect. The oil body may also disintegrate prior to the exertion of the biological effect by the active ingredient.

The creamy texture of the oil bodies make embodiments of the invention where the composition is applied topically particularly preferred.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

Example 1

Preparation of Oil Bodies from Oilseed rape, Soybean, Sunflower, White Mustard, Peanut, Squash, Flax, Safflower and Maize.

Dry mature seeds obtained from *Brassica napus* cv Westar (oilseed rape), soybean, sunflower, white mustard, peanut, squash, flax, safflower and maize were homogenized in five volumes of cold grinding buffer (50 mM Tris-HCl, pH 7.5, 0.4 M sucrose and 0.5 M NaCl) using a polytron operating at high speed. The homogenate was centrifuged at 10 x g for 30 minutes in order to remove particulate matter and to separate oil bodies from the aqueous phase containing the bulk of the soluble seed protein. The oil body fraction was skimmed from the surface of the supernatant with a metal spatula and added to one volume of grinding buffer. In order to achieve efficient washing in subsequent steps it was found to be necessary to thoroughly redisperse the oil bodies in the grinding buffer. This was accomplished by gently homogenizing the oil bodies in grinding buffer using a

polytron at low speed. Using a syringe, the redispersed oil bodies were carefully layered underneath five volumes of cold 50 mM Tris-HCl pH 7.5 and centrifuged as above. Following centrifugation, the oil bodies were removed and the washing procedure was repeated two times. The final washed oil body preparation was resuspended in one volume of cold Tris-HCl pH 7.5, redispersed with the polytron.

The oil body samples were dissolved in SDS sample buffer and protein profiles for the oil body samples unique to each of the plant species were obtained following SDS gel electrophoresis. The results are shown in Fig. 1.

Example 2

The Preparation of Oil Bodies from Oilseed Rape, Sunflower and Maize on a Large Scale.

Grinding of seeds. A total of 10 - 15 kgs of dry canola seed (*Brassica napus* cv Westar), sunflower (*Helianthus annuus*) or maize (*Zea mays*) was poured through the hopper of a colloid mill (Colloid Mill, MZ-130 (Fryma); capacity: 500 kg/hr), which was equipped with a MZ-120 crosswise toothed rotor/stator grinding set and top loading hopper. Approximately 50 - 75 l water was supplied through an externally connected hose prior to milling. Operation of the mill was at a gap setting of 1R, chosen to achieve a particle size less than 100 micron at 18°C and 30°C. Following grinding of the seeds tap water was added to the seed slurry to a final volume of 90 litres.

Removal of solids. The resulting slurry, was pumped into a decantation centrifuge (Hasco 200 2-phase decantation centrifuge maximum operating speed 6,000 rpm) after bringing the centrifuge up to an operating speed of 3,500 rpm. Transfer from the mill to the decantation centrifuge at a flow rate of 360 L/hr was achieved using a 1 inch M2 Wilden air operated double diaphragm pump. In 15-20 minutes approximately 15 kg of seed was decanted.

Oil body separation. Separation of the oil body fraction was achieved using a Sharples Tubular Bowl Centrifuge model AS-16 (Alpha Laval) equipped with a three phase separating bowl and removable ring dam series; capacity:150 L/hr; ringdam: 30 mm. Operating speed was at 15,000 rpm (13,200 x g). A Watson-Marlow (Model 704) peristaltic pump was used to pump the decanted liquid phase (DL) into the tubular bowl centrifuge after bringing the centrifuge up to operating speed. This results in separation of the decanted liquid phase into a heavy phase (HP) comprising water and soluble seed proteins and a light phase (LP) comprising oil bodies. The oil body fraction which was obtained after one pass through the centrifuge is referred to as an unwashed oil body preparation. The oil body fraction was then passed through the centrifuge three more times. Between each pass through the centrifuge, concentrated oil bodies were mixed with approximately five volumes of fresh water. The entire procedure was carried out at room temperature. The preparations obtained following the second separation are all referred to as the washed oil

body preparation. Following three washes much of the contaminating soluble protein was removed and the oil body protein profiles obtained upon SDS gel electrophoresis were similar in appearance to those obtained using laboratory scale procedures (Example 1).

Example 3

Comparison of Washed Oil Bodies and Lipid Vesicles with respect to Utility as an Ingredient for the Preparation of Formulations Acceptable for Topical Delivery to Humans. Washed Oil bodies were prepared as described in example 2 using safflower seed, pasteurized and 0.1% BHT, 0.1% BHA and 0.1% Glydant plus added. Lipid vesicles were prepared in accordance with the specification of US Patent 5,683,740 except that they were prepared from safflower seed and pasteurized and 0.1% BHT, 0.1% BHA and 0.1% Glydant Plus were added.

Oil bodies and lipid vesicles were compared with respect to emulsion stability, color changes, odor changes, viscosity, microbial growth and cosmetic desirability parameters. To evaluate stability, the samples were tested at 45°C, 4°C and room temperature (3 months at 45°C is equivalent to approximately 2 year shelf life at room temperature). To evaluate emulsion stability, 150 g of each sample was maintained at 45°C, 75 g of each sample was maintained at room temperature or at 4°C. Emulsion stability was evaluated for emulsion separation, oil droplet separation and coalescence. The 4°C sample was used as the reference for comparison. Color changes were evaluated by visual inspection. Color was evaluated on the accelerated oven sample (45°C) and the room temperature sample and compared to the 4°C as a reference. Odor was tested as with the color with the 4°C sample used as a reference point. In order to maintain consistency, the odor was judged by two individuals who both agreed on the evaluation. Viscosity of each sample was measured at room temperature using a RVT Model viscometer with Spindle E at 10rpm. Microbial growth was measured on 10 g of each sample. The sample was diluted and 1 ml of the sample is added to 49°C Tryptic Soy Agar, swirled and allowed to cool. The plates were incubated at 35°C for 48 hours and a colony count was taken. Finally, cosmetic attributes were evaluated by 3 individuals, 2 individuals who were familiar with oil bodies/lipid vesicles and 1 person who was not. Cosmetic attributes include skin penetration, residue left on the skin after the sample was rubbed in, dryness (lack of moisture) and oiliness.

Table 1 summarizes the results for the oil bodies. The pH for the oil body sample was constant at 6.50 throughout the test at room temperature and at 45°C. The oil body preparation, when applied to the skin, distributed evenly on the skin, was fast penetrating and left almost no residue on the skin surface. The oil body preparation was also stable with respect to color, odor, viscosity and emulsion stability.

Table 2 summarizes the results for the lipid vesicles. The pH for the lipid vesicle sample is difficult to measure because of the total separation but was

approximately 6.8. The lipid vesicle preparation, when applied to the skin, was very oily and left a film residue on the skin. The lipid vesicle preparation was stable with respect to microbial growth but was not stable with respect to color, odor and emulsion stability.

Thus the oil washed oil body preparation is clearly superior to lipid vesicles with respect to many parameters including the following parameters, color, odor, stability and cosmetic parameters like penetration, residual residue, and oiliness, which are critical to utility as a delivery vehicle.

Example 4

Preparation of Compositions for Topical Delivery and Irritation reactions to Oil Bodies and the Effect of Oil Bodies on Known Dermal Irritants. The following formulations were used in an irritation study to test whether or not oil bodies cause irritation and whether or not oil bodies had an effect on known irritants used in cosmetic formulations (e.g. hydroquinone, alpha hydroxy acid and salicyclic acid). The formulation was mixed to form an emulsion with the following procedure. Phase I is the water phase. In this phase, the water in the main tank is charged. A propeller is used to hydrate the sodium thiosulfate, glycolic acid and/or salicyclic acid, if added to the formulation, with moderate agitation at room temperature. The water phase is heated to a final temperature of 75°C to 77°C. Phase II, the oil phase is mixed in a separate mixing pot with moderate agitation and then subsequently heated up to 75°C to 77°C. The oil phase ingredients include Keltrol, Arlacel 165 and Glydant Plus. The final step of emulsification includes the addition of the oil phase (Phase II) to the water phase (Phase I). The two phases are mixed under high agitation with a propeller or homogenizer for 15 minutes. After 15 minutes of mixing the mixture is cooled slowly to 50°C. If sodium thiosulfate and/or lactic acid is/are added, they are added at 50°C. The agitation is decreased as the temperature decreases. At 40°C the Hydroquinone Liposomized (25% HQ) and/or Hydroquinone is added and when the temperature reaches about 37°C to 40°C the safflower oil bodies are added slowly.

Formulation A

Hydrated safflower oil bodies (0.1% Glydant plus, 0.1% BHT, 0.1% BHA)	100.00%

Formulation B

Distilled Water	43.85%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.1%
Hydrated safflower oil bodies (0.1% Glydant plus, 0.1% BHT, 0.1% BHA)	49.00%
Hydroquinone	2.00%
Sodium thiosulfate	0.30%
Lactic acid	0.17%
pH	4.00
Viscosity, RVT E/10rpm (cps)	50,000

Formulation C

Distilled Water	68.04%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Hydroquinone	2.00%
Sodium thiosulfate	0.30%
Safflower oil	25.00%
Lactic acid	0.06%
pH	4.17
Viscosity, RVT E/10rpm (cps)	35,000

Formulation D

Distilled Water	87.40%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Hydroquinone Liposomized (25%)	8.00%
Sodium thiosulfate	0.30%
Lactic acid	0.06%
pH	5.38
Viscosity, RVT E/10rpm (cps)	15,000

Formulation E

Distilled Water	93.10%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Hydroquinone	2.00%
Sodium thiosulfate	0.30%
pH	4.17
Viscosity, RVT E/10rpm (cps)	22,000

Formulation F

Distilled Water	46.34%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Hydrated safflower oil bodies (0.1% Glydant plus, 0.1% BHT, 0.1% BHA)	49.00%
Lactic acid	0.06%
pH	4.60
Viscosity, RVT E/10rpm (cps)	20,000

Formulation G

Distilled Water	41.34%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Hydrated safflower oil bodies (0.1% Glydant plus, 0.1% BHT, 0.1% BHA)	46.00%
Lactic acid	0.06%
Glycolic acid (AHA)	8.00%
pH	4.00
Viscosity, RVT E/10rpm (cps)	50,000

Formulation H

Distilled Water	87.34%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Lactic acid	0.06%
Glycolic acid (AHA)	8.00%
pH	2.09
Viscosity, RVT E/10rpm (cps)	7,500

Formulation I

Distilled Water	44.34%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Hydrated safflower oil bodies (0.1% Glydant plus, 0.1% BHT, 0.1% BHA)	49.00%
Lactic acid	0.06%
Salicyclic acid	2.00%
pH	3.00
Viscosity, RVT E/10rpm (cps)	27500

Formulation J	
Distilled Water	93.34%
Keltrol	1.50%
Arlacel-165	3.00%
Glydant Plus	0.10%
Lactic acid	0.06%
Salicyclic acid	2.00%
pH	2.87
Viscosity, RVT E/10rpm (cps)	10,000

Formulation K	
Sodium Lauryl Sulfate	0.1% w/v

Formulation L	
Saline	0.9%

In order to test the irritation reaction of oil bodies and oil bodies with active ingredients, a sufficient number of subjects were enrolled so that 25 test subject completed the study. Each test subject was tested with the 12 formulations (A to L) in a random manner in order to not introduce any bias. 0.2 mL of each formulation was applied to the paraspinal region of the back of each subject using a Eppendorf repeater pipette. Formulations A though J and L were covered by a semi-open occluded patch with adhesive removed from two opposing sides. Formulation K (the positive control which was known to cause irritation) was covered with a occluded patch using a nonwoven cotton pad (Webril®) covered by and secured on all sides by hypoallergenic tape (e.g. Blenderm™). The assignment of test articles to individual skin sites were rotated so that each test article occupies individual skin sites within the panel of test subjects, with approximately equal frequency, in order to eliminate any position bias. The individual test articles were applied to the skin for contact periods of approximately 23 hours per application. After 23 hours, the subject removed the patch, bathed or showered and reported for scoring. Application was made every day for fourteen consecutive days on the same site unless reaction to any of the formulations made this inadvisable. Irritation was scored as per Berger and Bowman. 1984. J. Toxicology Cut. And Ocular Toxicol 1: 109-115. Scoring was conducted using a 100 watt incandescent blue bulb lamp. The scorer was blinded as to the

treatment assignments and to any previous scores. A reasonable attempt was made to ensure that the same individual did all of the scoring.

Scores were expressed as the Total Score for a base of 10 subjects and classified according to the following empirically derived categorization system. A score of 0-33 indicates that the material is mild and that there was not experimental irritation, 34-133 indicates that the material is probably mild in normal use as there was evidence of a slight potential for mild cumulative irritation, a score of 134-299 is indicative of the material being possibly mild in normal use as there was some evidence of a moderate potential for mild cumulative irritation, a score in the of 300-387 indicates an experimental cumulative irritant with a strong potential for mild to moderate cumulative irritation and finally, a score of 388-420 indicates that experimental primary irritant with evidence of potential for primary irritant irritation. The scores for formulations A through L are presented in Table 3.

Formulation K is used as the positive control with a high level of irritation and formulation L is the negative control demonstrating no experimental irritation. The lowest level of irritation was found in formulation A and F which are oil bodies alone and formulated oil bodies, respectively. This level of irritation was even lower than the saline control (formulation L). A substantial decrease in irritation was observed when hydroquinone was formulated with oil bodies (formulation B) when compared to hydroquinone formulations with safflower oil (formulation C), hydroquinone in liposomes (formulation D) or hydroquinone formulated alone (formulation E). Similarly there was a decrease in the irritation caused by salicyclic acid when the salicyclic acid was formulated with oil bodies (Formulation I) when compared to salicyclic acid formulated without oil bodies (formulation J). There was little difference with the alpha hydroxy acid formulated with and without oil bodies, in formulations G and H respectively. This is likely due to the fact that both formulations were occluded and this is known to be undesirable with AHA.

Thus these results indicate that oil bodies decrease the irritation caused by active ingredients like hydroquinone (bleaching agent) and salicyclic acid (exfolient).

Example 5

Detection of Oil Body-Associated Protein Penetration in Human Skin Samples

Preparation of Oil Bodies. Washed oil bodies were prepared aseptically from seeds of non-transgenic canola using standard laboratory procedures. Extraction and washing steps were performed with sterile water. The final percentage dry weights of the oil body preparations was approximately 60%.

Franz cell methodology. Human cadaver skin from a single donor was dermatomed to approximately 200 micro thickness. Eighteen Franz static diffusion glass diffusion chambers (Crown Glass Cat # FDC-100) with a magnetic stirrer mounted on a 9-

position Franz diffusion cell drive console with acrylic blocks, magnetic stirrers, and stainless steel manifolds (Crown Glass Cat #FDCD-9-LV) were used for the Franz cell assay. The chambers were filled with isotonic buffered saline (pH 7.4) and equilibrated for 1 to 2 hours to a temperature of 37°C by a circulating Haake water pump prior to applying the skin specimen. Duplicate samples of two types of skin specimens were tested: dermatomed intact skin tape-stripped 24 hours after applying formulation and dermatomed tape-stripped skin stripped before applying the formulation (Tape-strip skin with cellophane tape until "glistening" (approximately 22 strips) or until epidermal separation starts to occur). The skin sample was placed on the chamber and sealed with an O-ring. The exposed skin surface area is 1.77 cm2. 20 mg of the test formulation (either wild type oil bodies) was applied to the skin surface with a Gilson P100 Pipetteman→ micropipet. A reservoir solution was collected after 1 hours and 24 hours after application of formulation. After 24 hours the skin surface was washed three times with 1.0 ml 2% Oleth-20 (Croda, Inc. #9004-98-2) in water. After washing, the skin was wiped with 3 sequential cotton gauze cloths. The skin was removed from the chamber. The dermatomed intact skin that was intended to be tape-stripped was tape-stripped. For all of the tape-stripped specimens (both pre-stripped and post-stripped samples), the dermis and epidermis were separated by placing the sample on a 60°C hot plate for one minute. The epidermis and dermis tissues were collected in separate vials and refrigerated.

Detection of oil body associated proteins. Tissue samples (intact skin, stripped skin, epidermis, dermis) were extracted with approximately 0.5 ml of SDS extraction buffer (2% SDS, 50 mM Tris-HCl, ph 7.5, 1 mM PMSF) by grinding with mortar and pestle. Reservoir samples from the Franz cells were made up to 2% SDS by addition of 1/10 volume of a 20% SDS stock solution. All samples were heated in a boiling water bath for 5 minutes and then frozen at -20°C until required. Protein determinations were made using the BCA method.

Protein samples were subsequently separated through SDS PAGE and electroblotted to PVDF membrane. A sample of 25 ug of protein from control epidermis samples spiked with 0.2 ug of canola oil body protein was included on each gel to enable relative quantification of protein penetration in treated tissue samples. Blots were probed with either anti-oleosin (*B. napus*) followed by anti-rabbit IgG antibodies conjugated to alkaline phosphatase. Cross-reacting protein was visualized by NBT / BCIP assay for alkaline phosphatase activity.

Results for the epidermal and dermal fractions wherein the stratum corneum was removed with skin stripping before the oil bodies were applied are shown in Figure 2. In this example the epidermal fractions from 3 individuals (lanes 3 through 5) and dermal fractions from the same 3 individuals (lanes 6 through 8) are compared to an epidermal extract spiked with 0.2 µg of canola oil body protein (lane 1) and control intact skin (lane 2).

The anti-oleosin antibody used in this experiment was a polyclonally-derived antibody to an canola oil body. As a result this anti-body will detect both oleosin protein and any proteins that are normally associated with canola oil bodies and were injected when antibodies were raised. In the epidermal fractions, oleosin as well as oil body associated proteins were detected in all individuals. In the dermis samples, oleosin was detected and to a lesser extent, oil bodies associated proteins were also detected. These results indicate that when oil bodies are applied to skin with the stratum corneum removed, oleosin and oil body associated proteins are able to penetrate into both the epidermis and dermis.

Results for the epidermal and dermal fractions wherein the stratum corneum was removed with skin stripping after the oil bodies were applied and the Franz assay was completed are shown in Figure 3. In this example the epidermal fractions from 3 individuals (lanes 2 through 4) and dermal fractions from the same 3 individuals (lanes 5 through 7) are compared to an epidermal extract spiked with 0.2 µg of canola oil body protein (lane 8) and control intact skin (lane 1). As in figure 2, the anti-oleosin antibody used in this experiment was a polyclonally-derived antibody to an canola oil body. Therefore this anti-body will detect both oleosin protein and any proteins that are normally associated with canola oil bodies. In the epidermal fractions, oleosin and oil body associated proteins were detected in all individuals, but with variation between individuals. As well, in the dermis, oleosin was detected and to a lesser extent, oil bodies associated proteins were also detected. These results indicate that when oil bodies are applied to skin with an intact stratum corneum, oleosin and oil body associated proteins are still able to penetrate into both the epidermis and dermis.

Taken together, results from figures 2 and 3 indicate that oleosin and oil body associated proteins are able to penetrate into both the epidermis and dermis. As well, in the presence of intact skin, oleosin and oil body associated proteins are able to traverse through the stratum corneum and penetrate into both the epidermis and dermis.

Example 6

Preparation of Compositions for the Delivery of Cosmetic Active Agents and Percutaneuous Absorption of Actives in Human Skin.

Preparation of Hydroquinone Formulations. A washed oil body preparation was prepared from safflower as in example 2. To the washed oil body preparation was added: 0.1% Glydant Plus, 0.1% Butylated Hydroxyanisole (BHA) 0.1% Butylated Hydroxytoluene) and a hydroquinone oil body formulation for use in a testing oil body-associated hydroquinone penetration was prepared as follows. Phase I is the water phase. In this phase, the water in the main tank is charged. A propeller is used to hydrate the Keltrol and Glydant plus with moderate agitation at room temperature. The glycerin is then added with continued mixing. The water phase is heated to a final temperature of 76°C to 78°C. Phase II, the oil phase is mixed in a separate mixing pot with moderate

agitation and then subsequently heated up to 76 to 78°C. The oil phase ingredients include, Arlacel 165, Cetyl Alcohol, Finsolv TN, and Permethyl 101A (Isohexadecane). The final step of emulsification includes the addition of the oil phase (Phase II) to the water phase (Phase I). The two phases are mixed under high agitation with a propeller or homogenizer for 15 minutes. After 15 minutes of mixing the mixture is cooled slowly to 40°C. The agitation is decreased as the temperature decreases. At approximately 40°C hydrated safflower oil bodies and hydroquinone are added slowly. The mixture is allowed to cool to room temperature. Citric Acid is added to the formulation until the pH is 3.5 to 4.0.

Oil body-hydroquinone formulation

Purified Water	36.1%
Keltrol	0.7%
Glycerin	2.0%
Glydant Plus	0.20%
Arlacel 165	3.0%
Cetyl Alcohol	2.0%
Finsolv TN	2.0%
Permethyl 101A	2.0%
Hydrated Safflower Oil Body (0.1% Glydant Plus, 0.1% BHT, 0.1% BHA)	50.0%
Hydroquinone	2.0%
Citric Acid to pH	3.5-4.0

A simple emulsion hydroquinone formulation for use in a testing a placebo-associated hydroquinone penetration was prepared as follows. Phase I is the water phase. In this phase, the water in the main tank is charged. A propeller is used to hydrate the Keltrol and Glydant plus with moderate agitation at room temperature. The glycerin is then added with continued mixing. The water phase is heated to a final temperature of 76°C to 78°C. Phase II, the oil phase is mixed in a separate mixing pot with moderate agitation and then subsequently heated up to 76 to 78°C. The oil phase ingredients include, Arlacel 165, Cetyl Alcohol, Finsolv TN, Permethyl 101A and (Isohexadecane). The final step of emulsification includes the addition of the oil phase (Phase II) to the water phase (Phase I). The two phases are mixed under high agitation with a propeller or homogenizer

for 15 minutes. After 15 minutes of mixing the mixture is cooled slowly to 40°C. The agitation is decreased as the temperature decreases. At approximately 40°C hydroquinone is added slowly. The mixture is allowed to cool to room temperature. Citric Acid is added to the formulation until the pH is 3.5 to 4.0.

Simple emulsion-hydroquinone formulation

Purified Water	87.2%
Keltrol	0.7%
Glycerin	2.0%
Glydant Plus	0.20%
Arlacel 165	3.0%
Cetyl Alcohol	2.0%
Finsolv TN	2.0%
Permethyl 101A	2.0%
Hydroquinone	2.0%
Citric Acid to pH	3.5-4.0

Preparation of Salicyclic Acid Formulations. A washed oil body preparation was prepared from safflower as in example 2. To the washed oil body preparation was added: 0.1% Glydant Plus, 0.1% Butylated Hydroxyanisole (BHA) 0.1% Butylated Hydroxytoluene) and a salicyclic acid oil body formulation for use in a testing oil body-associated salicyclic penetration was prepared as follows. Phase I is the water phase. In this phase, the water in the main tank is charged. A propeller is used to hydrate the Keltrol and Glydant plus with moderate agitation at room temperature. The glycerin is then added with continued mixing. The water phase is heated to a final temperature of 76°C to 78°C. Phase II, the oil phase is mixed in a separate mixing pot with moderate agitation and then subsequently heated up to 76 to 78°C. The oil phase ingredients include, Arlacel 165, Cetyl Alcohol, Finsolv TN, and Permethyl 101A (Isohexadecane). The final step of emulsification includes the addition of the oil phase (Phase II) to the water phase (Phase I). The two phases are mixed under high agitation with a propeller or homogenizer for 15 minutes. After 15 minutes of mixing the mixture is cooled slowly to 40°C. The agitation is decreased as the temperature decreases. At approximately 40°C hydrated safflower oil bodies and salicyclic acid are added slowly. The mixture is allowed to cool to room temperature. Citric Acid is added to the formulation until the pH is 3.5 to 4.0.

Oil body-salicyclic acid formulation

Purified Water	36.1%
Keltrol	0.7%
Glycerin	2.0%
Glydant Plus	0.20%
Arlacel 165	3.0%
Cetyl Alcohol	2.0%
Finsolv TN	2.0%
Permethyl 101A	2.0%
Hydrated Safflower Oil Body (0.1% Glydant Plus, 0.1% BHT, 0.1% BHA)	50.0%
Salicyclic Acid	2.0%
Citric Acid to pH	3.5-4.0

A simple emulsion salicyclic acid formulation for use in a testing placebo-associated salicyclic acid penetration was prepared as follows. Phase I is the water phase. In this phase, the water in the main tank is charged. A propeller is used to hydrate the Keltrol and Glydant plus with moderate agitation at room temperature. The glycerin is then added with continued mixing. The water phase is heated to a final temperature of 76°C to 78°C. Phase II, the oil phase is mixed in a separate mixing pot with moderate agitation and then subsequently heated up to 76 to 78°C. The oil phase ingredients include, Arlacel 165, Cetyl Alcohol, Finsolv TN, Permethyl 101A and (Isohexadecane). The final step of emulsification includes the addition of the oil phase (Phase II) to the water phase (Phase I). The two phases are mixed under high agitation with a propeller or homogenizer for 15 minutes. After 15 minutes of mixing the mixture is cooled slowly to 40°C. The agitation is decreased as the temperature decreases. At approximately 40°C salicyclic acid is added slowly. The mixture is allowed to cool to room temperature. Citric Acid is added to the formulation until the pH is 3.5 to 4.0.

Simple emulsion-salicyclic acid formulation

Purified Water	85.3%
Keltrol	0.7%
Glycerin	2.0%
Glydant Plus	0.20%
Arlacel 165	3.0%
Cetyl Alcohol	2.0%
Finsolv TN	2.0%
Permethyl 101A	2.0%
Salicyclic acid	2.0%
Citric Acid to pH	3.5-4.0

Preparation of Tretinoin Formulations. A washed oil body preparation was prepared from safflower as in example 2. To the washed oil body preparation was added: 0.1% Glydant Plus, 0.1% Butylated Hydroxyanisole (BHA) 0.1% Butylated Hydroxytoluene (BHT) and a tretinoin oil body formulation for use in a testing oil body-associated tretinoin penetration was prepared as follows. Phase I is the water phase. In this phase, the water in the main tank is charged. A propeller is used to hydrate the Keltrol and Glydant plus with moderate agitation at room temperature. The glycerin is then added with continued mixing. The water phase is heated to a final temperature of 76°C to 78°C. Phase II, the oil phase is mixed in a separate mixing pot with moderate agitation and then subsequently heated up to 76 to 78°C. The oil phase ingredients include, Arlacel 165, Cetyl Alcohol, Finsolv TN, and Permethyl 101A (Isohexadecane). The final step of emulsification includes the addition of the oil phase (Phase II) to the water phase (Phase I). The two phases are mixed under high agitation with a propeller or homogenizer for 15 minutes. After 15 minutes of mixing the mixture is cooled slowly to 40°C. The agitation is decreased as the temperature decreases. At approximately 40°C hydrated safflower oil bodies and tretinoin are added slowly. The mixture is allowed to cool to room temperature. Citric Acid is added to the formulation until the pH is 3.5 to 4.0.

Oil body-tretinoin formulation

Purified Water	38.0%
Keltrol	0.7%
Glycerin	2.0%
Glydant Plus	0.20%
Arlacel 165	3.0%
Cetyl Alcohol	2.0%
Finsolv TN	2.0%
Permethyl 101A	2.0%
Hydrated Safflower Oil Body (0.1% Glydant Plus, 0.1% BHT, 0.1% BHA)	50.0%
Tretinoin	0.10%
Citric Acid to pH	3.5-4.0

A simple emulsion tretinoin formulation for use in a testing placebo-associated tretinoin penetration was prepared as follows. Phase I is the water phase. In this phase, the water in the main tank is charged. A propeller is used to hydrate the Keltrol and Glydant plus with moderate agitation at room temperature. The glycerin is then added with continued mixing. The water phase is heated to a final temperature of 76°C to 78°C. Phase II, the oil phase is mixed in a separate mixing pot with moderate agitation and then subsequently heated up to 76 to 78°C. The oil phase ingredients include, Arlacel 165, Cetyl Alcohol, Finsolv TN, Permethyl 101A and (Isohexadecane). The final step of emulsification includes the addition of the oil phase to the water phase. The two phases are mixed under high agitation with a propeller or homogenizer for 15 minutes. After 15 minutes of mixing the mixture is cooled slowly to 40°C. The agitation is decreased as the temperature decreases. At approximately 40°C tretinoin is added slowly. The mixture is allowed to cool to room temperature. Citric Acid is added to the formulation until the pH is 3.5 to 4.0.

Simple emulsion-tretinoin formulation

Purified Water	85.3%
Keltrol	0.7%
Glycerin	2.0%
Glydant Plus	0.20%
Arlacel 165	3.0%
Cetyl Alcohol	2.0%
Finsolv TN	2.0%
Permethyl 101A	2.0%
Tretinoin	0.10%
Citric Acid to pH	3.5-4.0

Detection of cosmetic actives in skin samples. Skin-stripping was used to evaluate the percutaneous absorption of the therapeutic actives across human skin by removing the individual cellular layers. The formulations were applied to the arm, which had been cleaned and left to dry for 10 minutes. Four circles 2 cm in diameter are marked on the skin and forty µl of product was applied and exposed to open air for 3 hours. After 3 hours, a 2 cm wide semi-transparent 3M piece of tape was applied on the skin under constant application pressure and removed with one swift motion. The strip was placed into a 5 ml screw top tube and 1 ml of methanol was added to the tube, vortexed for one minute and put in a rack. The extraction was allowed to proceed for at least 15 minutes. Once extraction was complete, an aliquot volume was taken for injection on a HPLC Columbus 250X4.6 mm CB column. Concentration of the active ingredient was determined for each strip by comparison to the area under the curve of a known standard tested on the same column.

Figure 4 is a histogram representing the cumulative results of 7 strips in a skin-stripping assay for formulations containing salicyclic acid, hydroquinone and tretinoin. Compared are the oil body formulations containing the active ingredient and a simple emulsion placebo containing the active ingredient. The cumulative percentage recovery of active ingredient obtained from 7 strips is indicated. As shown in figure 4, all active ingredients tested (salicyclic acid, hydroquinone and tretinoin) were shown to have a higher level of penetration (lower percent recovery) when compared to the placebo emulsion formulation with the same active ingredient. In particular, a higher level of penetration was observed for the oil body based formulation beyond the stratum corneum.

TABLE 1

Room Temperature					
Time (days)	**Color**	**Odor**	**Stability**	**Viscosity (cps)**	**Microbial growth**
0	Pale yellow	Very mild	No separation	3500 +/- 100	500
14	Pale yellow	No change	No separation	3500 +/- 100	300
25	Pale yellow	No change	No separation	3500 +/- 100	<10
45°C					
Time (days)	**Color**	**Odor**	**Stability**	**Viscosity (cps)**	**Microbial growth**
0	Pale yellow	Very mild	No separation	3500 +/- 100	500
14	Pale yellow	Mild	No separation	4000 +/- 100	<20
25	Mildly yellow	Mild	No separation	4000 +/- 100	<10
4°C					
Time (days)	**Color**	**Odor**	**Stability**	**Viscosity (cps)**	**Microbial growth**
0	Pale yellow	Very mild	No separation	3500 +/- 100	500
14	Pale yellow	Very mild	No separation	3500 +/- 100	250
25	Pale yellow	Very mild	No separation	3500 +/- 100	<10

TABLE 2

Room Temperature					
Time (days)	**Color**	**Odor**	**Stability**	**Viscosity (cps)**	**Microbial growth**
0	Dark yellow	Very mild	Separation	Approx 4000	<20
14	Dark yellow	Very mild	Total separation	Sluggish	<20
25	Darker yellow	Very mild	Total separation	Sluggish	<10
45°C					
Time (days)	**Color**	**Odor**	**Stability**	**Viscosity (cps)**	**Microbial growth**
0	Dark yellow	Neutral	Separation	Approx. 4000	<20
14	Brown	Amine odor	Total separation	Sluggish	<10
25	Dark brown	Fishy	Total separation	Sluggish	<10
4°C					
Time (days)	**Color**	**Odor**	**Stability**	**Viscosity (cps)**	**Microbial growth**
0	Dark yellow	Neutral	Separation	Approx. 4000	<20
14	Dark yellow	Neutral	Total separation	Sluggish	<10
25	Dark yellow	Neutral	Total separation	Sluggish	<10

TABLE 3

Formulation	Score	Classification
A	8.8	Mild material – no experimental irritation
B	56.5	Probably mild in normal use
C	174.1	Possibly mild in normal use
D	160.7	Possibly mild in normal use
E	38.5	Possibly mild in normal use
F	5.4	Mild material – no experimental irritation
G	377.9	Experimental cumulative irritant
H	373.2	Experimental cumulative irritant
I	156.0	Possibly mild in normal use
J	211.4	Possibly mild in normal use
K	295.1	Possibly mild in normal use
L	25.8	Mild material – no experimental irritation

WE CLAIM:

1. A composition for the improved topical delivery of an active agent which comprises:

(1) an active agent; and

(2) oil bodies.

2. A composition according to claim 1 wherein said active agent is a cosmetically active agent or a dermatologically active agent.

3. A composition according to claim 1 wherein said oil bodies are obtained from plant cells.

4. A composition according to claim 1 wherein said active agent is an anti-aging agent, a sunscreen, a bleaching agent, an immunostimulatory agent, an exfolient or an anti-wrinkling agent.

5. A composition according to claim 1 wherein said active agent is hydroquinone, salicylic acid, retinyl palmitate, alpha-hydroxy acid, a vitamin, tretinoin or a protein.

6. A composition according to claim 1 wherein said active agent is a polypeptide linked to a sufficient portion of an oleosin to provide targeting of said polypeptide to an oil body.

7. A composition according to any one of claims 1 to 6 wherein said oil bodies are substantially intact.

8. A composition according to any one of claims 1 to 7 to wherein said oil bodies are washed.

9. A composition according to any one of claims 1 to 8 wherein the oil bodies are obtained by a method comprising:

(1) grinding plant seeds;

(2) removing solids from the ground seeds; and

(3) separating the oil body phase from the aqueous phase.

10. A composition according to claim 9 wherein said oil body phase comprises substantially intact oil bodies.

11. A composition according to claim 10 wherein said oil body phase is washed to yield a washed oil body preparation.

12. A composition according to any one of claims 1 to 8 wherein said oil bodies and active agent are obtained by:

(a) introducing into a host cell a chimeric DNA sequence comprising:

(1) a first DNA sequence capable of regulating the transcription in said host cell of

(2) a second DNA sequence, wherein said second sequence encodes a recombinant fusion polypeptide and comprises a DNA sequence encoding a sufficient portion of an oleosin protein to provide targeting of the recombinant fusion polypeptide to an oil body phase, linked in frame to (ii) a DNA sequence encoding the active agent; and

(3) a third DNA sequence encoding a termination region functional in said host cell; and

(b) growing said host cell to produce said recombinant fusion polypeptide, and

(c) separating the oil body fraction of said host cell from the cellular components to prepare oil bodies and the active agent.

13. A composition according to any one of claims 3 or 9 to 12 wherein said plant is selected from the group consisting of rapeseed (*Brassica* spp.), soybean (*Glycine max*), sunflower (*Helianthus annuus*), oil palm (*Elaeis guineeis*), cottonseed (*Gossypium* spp.), groundnut (*Arachis hypogaea*), coconut (*Cocus nucifera*), castor (*Ricinus communis*), safflower (*Carthamus tinctorius*), mustard (*Brassica* spp. and *Sinapis alba*), coriander (*Coriandrum sativum*), squash (*Cucurbita maxima*), linseed/flax (*Linum usitatissimum*), Brazil nut (*Bertholletia excelsa*), jojoba (*Simmondsia chinensis*), maize (*Zea mays*), crambe (*Crambe abyssinica*) and eruca (*Eruca sativa*).

14. A composition according to any one of claims 1 to 13 wherein the active agent is linked to the oil bodies.

15. A composition according to claim 14 wherein the active agent is covalently linked to the oil bodies.

16. A composition according to claim 15 wherein the active agent is non-covalently linked to the oil bodies.

17. A method of preparing a composition according to any one of claims 1 to 16 comprising formulating the active agent in the presence of the oil bodies.

18. A use of a composition comprising an active agent and oil bodies to prepare a medicament for the topical delivery of an active agent to a living organism.

19. A use according to claim 18 wherein said topical delivery results in percutaneous absorption of the active agent to the stratum corneum, the epidermis or the dermis.

20. A use according to claims 18 or 19 wherein said active agent is a cosmetically active agent or a dermatologically active agent.

21. A use according to any one of claims 18 to 20 wherein said active agent is an anti-wrinkling agent, an anti aging agent, a sunscreen, a bleaching agent, an immunostimulatory agent or an exfolient.

22. A use according to any one of claims 18 to 20 wherein said active agent is hydroquinone, salicylic acid, retinyl palmitate; alpha-hydroxy acid, a vitamin, tretinoin or a protein.

23. A use according to any one of claims 18 to 22 wherein said living organism is a mammal.

24. A use according to claim 23 wherein said mammal is a human.

25. A use according to any one of claims 18 to 24 wherein the oil bodies enhance percutaneous absorption or penetration of the active agent.

26. A use according to any one of claims 18 to 25 wherein the oil bodies reduce the irritability to the skin of the active agent.

27. A use of a composition according to any one of claims 1 to 16 to deliver an active agent or dermatologically active agent to an organism.

FIGURE 1

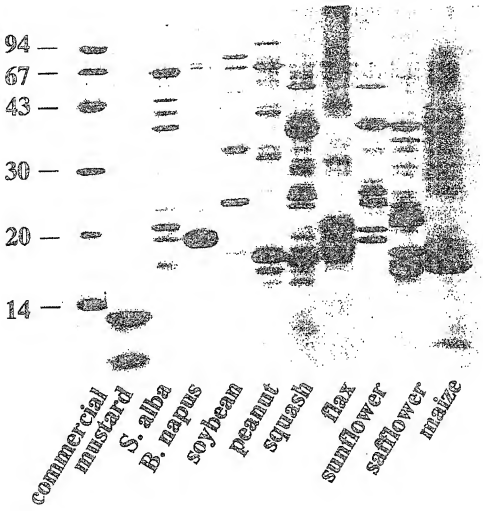

94
67
43
30
20
14
commercial mustard
S. alba
B. napus
soybean
peanut
squash
flax
sunflower
safflower
maize

FIGURE 2

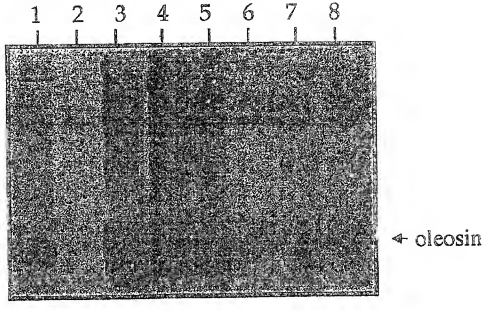


FIGURE 3

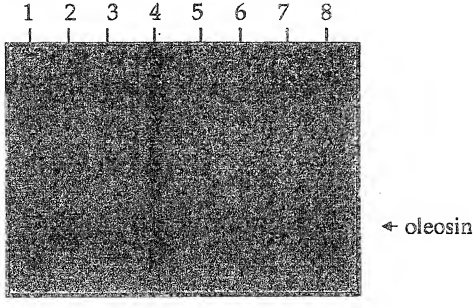
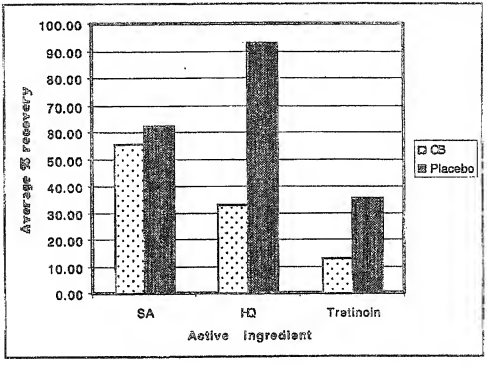


FIGURE 4


100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Average % recovery
SA
HQ
Tretinoin
Active Ingredient
CB
Placebo